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# Effect of Autologous Multiple Bone Marrow Aspirate on the Healing of Metacarpal and Metatarsal Fractures Reduced by Internal Fixators in Beetal Goats

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**Key words**
**Beetal goats, Bone marrow aspirate, Long bone fracture, Bone plating, Bone wiring**

## ABSTRACT

Fractures related to long bones in female Beetal goats fail to heal or show delayed healing that leads to intensified morbidity. Bone marrow aspirate (BMA) has been suggested as an efficient biological adjuvant for healing long bone fractures. BMA comprises bone mesenchymal stem cells. This study aims to assess the potential of autologous BMA on metacarpal and metatarsal fracture of Beetal goats presented at the surgery clinic of the University of Veterinary and Animal Sciences Lahore, Pakistan. Beetal goats were selected (n=20), and divided into four different groups. The first group was designated as bone plating with bone marrow aspirate (BPMA) in which fracture was reduced by using the bone plates along with the application of multiple BMA on days: 0, 14, 28, and 45. Furthermore, the second group was designated as bone plating with normal saline (BPNS) in which fracture was reduced by using the bone plates along with the application of normal saline. Additionally, the third group was designated as bone wiring with bone marrow aspirate (BWBM), and the fourth group, was bone wiring with normal saline (BWNS). Both third and fourth groups were treated with bone wiring along with BMA and bone wiring along with NS respectively. The rate of healing post-treatment was assessed by radiographic union score (RUS), weight-bearing score (WBS), and serobiochemical evaluations on days: 0, 7, 14, 28, and 45. Our data showed a significant difference in the healing of fractures treated with BMA as compared to NS on days; 7 and 14. Moreover, the RUS, WBS, and serobiochemical profiles of goats treated with BMA showed improved healing of fractures as compared to the goats treated with NS. In summary, we observed that the healing process of the metacarpal and metatarsal fractured bones was reduced by bone plating, and bone wiring was ameliorated with the application of multiple BMA. We proposed further studies on larger cohorts. BMA may be used as supportive therapy to enhance the healing process of fractures in goats.

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## INTRODUCTION

Fracture is the discontinuity of bone and is mostly associated with soft tissue damage, ruptured blood vessels, lacerated periosteum, and injured muscles and nerves (Doijode *et al.*, 2018). Trauma is the major cause of fracture (Ferrero *et al.*, 2022). Management of fracture revolves around restoration of function and physical integrity with the least deformity in bone (Slater and Mathen, 2021). For the treatment of fractures, different internal fixation methods like bone plating, bone wiring, intramedullary pinning, and compression screws are currently used in practice (Slater and Mathen, 2021).

Even though several veterinary surgeons are not satisfied with the results of internal fixators but it has been reported that internal fixators used in accordance with strict aseptic conditions, along with external immobilization and some additional support give much better results (Kurumurthy *et al*., 2021). Meanwhile, few studies mentioned that internal fixation is not preferred because of the long time required for the repair of fracture, follow on infections, failure of the implant, and bone deformity (Xie *et al*., 2021).

The use of osteogenic progenitor cell has been encouraged while treating several orthopedic conditions in animals. BMA is a good source of progenitor cells for skeletal tissues like cartilage and bone (Kumar *et al*., 2021). Recently, the use of BMA has acquired great attention (Jamal *et al*., 2022). It has been reported that BMA increases osteogenesis in bones during the repairing process of fracture (Antariksa, 2012; Kassem, 2013). Furthermore, the degree of healing in the fractured bone can be assessed clinically, radiographically, and by the use of bone turnover markers (Al-Sobayil *et al*., 2020).

To the best of our knowledge, no data is available regarding the treatment fractured bones of metacarpal and metatarsal by using internal fixators (bone plating and bone wiring) with BMA of and their effect on serum biochemistry in Beetal goats. Hence, our hypothesis was made on the basis of these contradictory results. We aim to investigate the effects of BMA on metacarpal and metatarsal fracture in goats reduced by bone plate and wire fixation based on RUS, WBS, and serobiochemical indicators.



## MATERIALS AND METHODS

*Animal selection*

A total of 20 clinical cases of long bone metacarpal and metatarsal fractures in Beetal goats presented to surgery clinic at the University of Veterinary and Animal Sciences (UVAS), Lahore that were selected for the study. All the selected animals were females with an average bodyweight of 22.5 and an age of 8-12 months. All animals were presented on the same day of metacarpal and metatarsal fracture occurrence. The study protocol was approved by the ethical review committee, UVAS, Lahore, Pakistan vide letter No. DR/27 date: 10$^{th}$ January 2019.

*Grouping of animals*

Beetal goats were selected (n=20), and divided into four different groups having five animals each. The first group was designated as bone plating with bone marrow aspirate (BPMA) in which fracture was reduced by using the bone plates along with the application of multiple BMA on days: 0, 14, 28, and 45. Furthermore, the second group was designated as bone plating with normal saline (BPNS) in which fracture was reduced by using the bone plates along with the application of normal saline. Additionally, the third group was designated as bone wiring with bone marrow aspirate (BWBM), and the fourth group, was bone wiring with normal saline (BWNS). Both third and fourth groups were treated with bone wiring along with BMA and bone wiring along with NS, respectively.

*Preoperative preparation and sedation*

Animals that had to undergo surgery have fasted for 12-h. The surgical site was prepared aseptically after clipping, shaving, and then scrubbing with an antiseptic solution of 5% povidone-iodine whereas, isopropyl alcohol was used as a degreasing agent. In all groups, animals were sedated with diazepam @ 0.25 mg/ kg body weight. After sedation induction was done with a combination of propofol and ketamine @ 2mg/kg body weight. After induction anesthesia was maintained with combination of diazepam @ 0.02 mg/kg/min + ketamine @ 0.04 mg/kg/min and propofol @ 0.016 mg/kg/min with constant rate of infusion using a volumetric syringe-driving pump

*Collection of bone marrow aspirate*

Animals were placed in lateral recumbency with the upward placement of the donor limb. The humerus was drawn cranially and the elbow was rotated towards the medial side. An incision was made between the greater tubercle and head of the humerus by using of sterna needle. At the base of the cortical bone, a puncture was made by slowly twisting the needle clockwise and anticlockwise. A 16-gauge needle was fitted with a 10 ml syringe and inserted into the bone marrow. After aspiration 3ml of bone marrow was centrifugated at 3200 rpm for 15 min. The resultant BMA was injected at the site of fracture after completion of surgery and then subcutaneously on the 14$^{th}$ and 28$^{th}$ days after surgery (Theophilus *et al*., 2018).

*Fracture repair: Bone plating with BMA (BPBM)*

The anesthetized goats were placed on right lateral recumbency and reduction of fracture was done by bone plating. Antiseptic was applied on the operative site and then draped. On the lateral side of the fractured bone, a skin incision was given distally from the proximal joint of the fractured bone up to above the distill joint for exposing the fractured site. Oblique fractures were found in the metacarpal and metatarsal bones. The fracture was reduced and held with the bone-holding clamp. Fractures were immobilized with dynamic compression plates placed on the lateral aspect of the fracture. Screws were fixed after drilling and tapping holes on both the cortices. The subcutaneous tissue was sutured using Vicryle No. 2 sutures in a simple continuous pattern. The skin was closed using monofilament Silk No. 2 sutures in a simple interrupted pattern as shown in Figure 1. The BMA was injected at the site of fracture immediately after surgery and then subcutaneously on the $14^{th}$ and $28^{th}$ days post-surgery.

Fig. 1. Application of bone plating and bone wiring in metatarsal bone fracture of female Beetal goats. Figure showed that the fractures of metatarsal bones in goats that is reduced by the application of bone plate and bone wiring.

*Bone plating with NS (BPNS)*

A similar surgical method as mentioned for animals of group BPBM was performed for animals of group BPNS, however, NS was injected at the site of fracture immediately instead of BMA and then at the $14^{th}$ and 28th-day post-surgery.

*Bone wiring with bone marrow (BWBM)*

Antiseptic was applied to the area for surgery and then draped. A skin incision was given on the lateral aspect of the fractured bone and reduction of fracture was done by bone holding clamps. After exposing the fracture site, wire sutures were used for internal fixation. Fractured bone fragments were immobilized with stainless steel wire using the full cerclage method. Equal tension was applied on both sides while wrapping wire around the bone after which two ends of the wire were united by twisting the wire. The subcutaneous tissue was sutured using Vicryle No. 2 sutures in a simple continuous pattern whereas, the skin was closed with monofilament Silk No. 2 sutures in a simple interrupted pattern as shown in Figure 1. The BMA was injected at the site of fracture immediately after surgery and then subcutaneously on the $14^{th}$ and $28^{th}$ days post-surgery.

*Bone wiring with NS (BWNS)*

A similar surgical method as mentioned for animals of group BWBM was performed for animals of group BWNS however, NS was injected at the site of fracture immediately instead of BMA and then at the $14^{th}$ and $28^{th}$-day post-surgery.

*Radiographic union score (RUS)*

For assessing the degree of recovery radiographs of fractured bone were obtained and graded according to the scoring system described by (Sandhu, 1987) which is as follows, 0 points when no evidence of callus formation; 1 point when callus formation occupies 25% of the gap; 2 points when callus formation occupies 50% of the gap; 3 points when callus formation occupies 75% of the gap; 4 points when callus formation occupying full gap formation.

*Weight-bearing score*

Weight-bearing was analyzed to assess the severity of lameness. On the day of presentation weight bearing grades of the affected limb was determined as mentioned by (Pierson, 2002) which are as follows:

0, Full weight bearing; 1, Weight bearing as tolerated; 2, Partial weight bearing; 3, Touch down weight bearing; 4, No weight bearing.

*Serobiochemical evaluation*

A needle of 16-gauge was placed intravenously in the left jugular vein of the goats. Five ml of blood samples were collected on 0 day pre-surgery and thereafter on days: 7, 14, 28, and 45 post-surgery. Serum was harvested and stored at -20°C until further analysis. Samples for analysis of calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), free hydroxyproline (FHP), and total hydroxyproline (THP) were stored under -20°C until further analyses. Samples for FHP and THP were analyzed with an automatic analyzer (Biosystems S.A. Costa Brava, Barcelona, Spain) whereas samples for Ca (MTD Diagnostics, IVD, Italy), P (Spectrum, SAE, Cairo, Egypt), and ALP (Sigma Aldrich® St. Louis, Missouri, USA) were analyzed using serum diagnostic kits.

*Statistical analysis*

The IBM SPSS version 20 (IBM Corp., Armonk, NY, USA) was utilized for statistical analyses. Mean ± standard deviation or median and ranges were employed to summarize quantitative data, whereas frequencies and percentages were used to organize qualitative data. Analysis of variance (ANOVA) was used to compare the mean difference of continuous variables between the groups (BPBM, BPNS, BWBM, and BWNS) while Mann Whitney U test was used to compare the median difference between the groups. The statistical significance was defined as a two-tailed p-value $< 0.05$.

## RESULTS

*Weight-bearing score*

In our dataset we observed that there was no significant difference in WBS in all animals on days; 0 and 7. However, the significant difference ($p < 0.05$) was observed in animals on days: 14, 28, and 45. Furthermore, we observed that the animals from groups BPBM and BWBM had better WBS compared to animals of BPNS and BWNS, respectively (Table I).

**Table I. Weight bearing score and radiographic union score of animals treated with bone plating and bone wiring along with bone marrow aspirate and normal saline.**

| Time | Group | | | | p value |
|---|---|---|---|---|---|
| | Median (Range) | | | | |
| | BPBM | BPNS | BWBM | BWNS | |
| **Weight bearing score** | | | | | |
| Day-0 | 4 (3 – 4) | 4 (3 – 4) | 4 (3 – 4) | 4 (4 – 4) | 0.759 |
| Day-7 | 3 (2 – 3) | 3 (3 – 4) | 3 (2 – 3) | 4 (3 – 4) | 0.048 |
| Day-14 | 2 (2 – 2) | 2 (2 – 3) | 2 (2 – 2) | 3(3 – 4) | 0.038 |
| Day-28 | 1 (1 – 1) | 2 (1 – 3) | 2 (1 – 2) | 3 (3 – 4) | 0.002 |
| Day-45 | 0 (0 – 1) | 1 (0 – 2) | 2 (1 – 2) | 3 (2 – 3) | 0.003 |
| **Radiographic union score** | | | | | |
| Day-0 | 0 (0 – 0) | 0 (0 – 0) | 0 (0 – 0) | 0 (0 – 0) | --- |
| Day-7 | 0 (0 – 1) | 0 (0 – 0) | 0 (0 – 1) | 0 (0 – 0) | 0.230 |
| Day-14 | 1 (1 – 2) | 1 (1 – 1) | 1 (0 – 2) | 0 (0 – 1) | 0.084 |
| Day-28 | 3 (2 – 3) | 2 (1 – 2) | 1 (1 – 3) | 1 (1 – 2) | 0.010 |
| Day-45 | 4 (3 – 4) | 3 (2 – 4) | 2 (1 – 3) | 1 (1 – 2) | 0.028 |

BPBM, Animals treated with bone plating and bone marrow aspirate; BPNS, Animals treated with bone plating and normal saline; BWBM, Animals treated with bone wiring and bone marrow aspirate; BWNS, Animals treated with bone wiring and normal saline; differences were considered significant at $p < 0.05$.

*Radiographic union score (RUS)*

Additionally, animals with BPBM had significantly higher ($p < 0.05$) RUS on days: 28 and 45 compared to animals of the other treatment groups. Furthermore, animals of groups BPBM and BWBM had better RUS compared to animals of BPNS and BWNS respectively (Table I).

*Serobiochemical evaluation*

Regarding the serum-biochemical profile of the selected parameters no significant difference was observed for Ca and P in all the animals. Moreover, it was observed that ALP and THP were significantly higher ($p < 0.05$) in the group BPBM compared with other groups on days: 14, 28, and 45. In addition, FHP was lower ($p < 0.05$) in group BWNS compared with other groups on days: 7, 14, 28, and 45 (Table II).

## DISCUSSION

For the development of new techniques for bone healing and accurate diagnosis of complications regarding bone fractures efforts are being made (Kurumurthy *et al*., 2021; Cox *et al*., 2010). Therefore, in this study the effect of bone marrow aspirate, weight-bearing score, radiographic union score, and serobiochemical markers were used for the assessment of bone healing, the selected parameters provide a favorable and simple noninvasive method for the evaluation of long bone fracture repair (El-Shafaey *et al*., 2014; Sousa *et al*., 2017).

During the observation period, a progressive improvement in weight bearing was noticed for all the animals. WBS was the same on day 0 among the groups while on day 7 a statistical difference ($P<0.05$) was observed among the groups: on day 28, day 14, and day 45. The results of the current study revealed that BPBM group showed better healing as compared to BPNS, BPBM, and BPNS groups. Al-Sobayil *et al*. (2020) identified the same results. This is due to the positive effect of bone marrow aspirate which has osteogenic stem cells and osteoconductive factors that are associated with an improved preparation of an inorganic scaffold that has been revealed to be a feasible regenerative system (Wu *et al*., 2022). This finding contributes the positive osteogenic properties demonstrated by the application of bone marrow aspirates (Al-Sobayil *et al*., 2020).

RUS improved outcomes were observed on day 28 and day 45 in animals. Furthermore, we identified that BPBM group showed better callus formation as compared to BPNS, BWBM, and BWNS groups. Similar results were noticed by (Al-Sobayil *et al*., 2020) which is due to the positive effect of bone marrow aspirate on the radiographic properties of newly formed callus at the fracture line. This positive effect is due to the delivery of connective tissue progenitors at concentrations that were slightly higher than what is found naturally in the bone marrow. This is the outcome of a significant increase in the rate of bone union of fracture (Muschler and Midura, 2002).

**Table II. Serobiochemical findings of animals treated with bone plating and bone wiring along with bone marrow aspirate and normal saline.**

| Parameter | Days | Treatments | | | | p value |
|---|---|---|---|---|---|---|
| | | BPBM | BPNS | BWBM | BWNS | |
| Phosphorus (mg/dl) | 0 | 7.22 ± 2.07 | 6.80 ± 1.95 | 7.34 ±2.24 | 7.14 ± 2.49 | 0.982 |
| | 7 | 8.84 ± 1.82 | 7.94 ± 1.81 | 8.84 ± 1.43 | 8.14 ±1.53 | 0.758 |
| | 14 | 9.20 ± 1.48 | 8.62 ± 1.05 | 9.44 ± 0.91 | 8.78 ± 0.90 | 0.644 |
| | 28 | 8.28 ± 1.59 | 8.06 ± 0.98 | 8.36 ± 0.77 | 8.58 ± 0.97 | 0.907 |
| | 45 | 7.54 ± 2.13 | 7.28 ± 1.61 | 7.56 ± 1.15 | 8.24 ± 1.11 | 0.792 |
| Calcium (mg/dl) | 0 | 8.84 ± 1.82 | 7.94 ± 1.81 | 8.84 ± 1.43 | 8.14 ± 1.53 | 0.758 |
| | 7 | 10.20 ± 0.83 | 9.80 ± 0.44 | 9.40 ± 1.14 | 9.40 ± 0.89 | 0.428 |
| | 14 | 11.60 ± 1.51 | 11.40 ± 0.54 | 10.80 ± 1.30 | 11.20 ± 0.83 | 0.710 |
| | 28 | 12.60 ± 0.54 | 12.40 ± 0.54 | 11.80 ± 0.83 | 11.80 ± 1.09 | 0.291 |
| | 45 | 9.40 ± 0.89 | 9.60 ± 0.54 | 9.80 ± 0.83 | 9.60 ± 0.54 | 0.891 |
| ALP (IU/L) | 0 | 113.20 ± 31.28 | 119.84 ±28.04 | 101.78 ±14.65 | 90.65 ± 12.39 | 0.240 |
| | 7 | 165.40 ± 37.55 | 154.06 ±30.21 | 147.90 ±22.40 | 120.34 ±14.61 | 0.105 |
| | 14 | 199.30 ± 42.16 | 181.83 ±41.71 | 204.93 ±25.23 | 143.42 ±15.77 | 0.040 |
| | 28 | 242.40 ± 54.30 | 196.54 ±28.67 | 241.69 ±29.51 | 134.40 ± 4.69 | 0.000 |
| | 45 | 254.62 ± 61.15 | 162.48 ±25.98 | 271.44 ±20.69 | 118.01 ±15.53 | 0.000 |
| FHP (mg/day) | 0 | 8.17 ± 0.12 | 8.20 ± 0.15 | 8.18 ± 0.11 | 8.16 ± 0.09 | 0.880 |
| | 7 | 9.06 ± 0.24 | 9.22 ± 0.14 | 8.84 ± 0.24 | 8.57 ± 0.14 | 0.001 |
| | 14 | 10.93 ± 0.21 | 10.996± 0.30 | 9.85 ± 0.63 | 9.58 ± 0.28 | 0.000 |
| | 28 | 11.48 ± 0.10 | 11.59 ± 0.22 | 10.69± 0.55 | 10.51 ± 0.26 | 0.000 |
| | 45 | 13.33 ± 0.24 | 12.87 ± 0.53 | 11.60 ± 0.74 | 11.35 ± 0.71 | 0.000 |
| THP (mg/day) | 0 | 10.25± 0.11 | 10.20 ± 0.15 | 10.18 ± 0.11 | 10.23 ± 0.04 | 0.786 |
| | 7 | 10.76 ± 0.27 | 10.50 ± 0.38 | 10.55 ± 0.44 | 10.39 ± 0.06 | 0.382 |
| | 14 | 11.25 ± 0.48 | 11.18 ± 0.26 | 10.46 ± 0.55 | 10.42 ± 0.28 | 0.007 |
| | 28 | 12.81 ± 0.58 | 12.50 ± 0.38 | 11.71 ± 0.95 | 11.54 ± 0.50 | 0.018 |
| | 45 | 15.19 ± 0.28 | 14.33 ± 1.13 | 12.79 ± 1.26 | 12.52 ± 0.86 | 0.001 |

For details see footnote of **Table 1**.



An increasing trend in the levels of serum Ca was revealed up to day 28 and then a decreasing trend on day 45 was found within groups. Increased levels of serum Ca in the initial levels are attributed to increased osteoclastic activity. Similar results were noticed by (Kumar, 2016) who used bone plates for the reduction of bone fractures in goats and also observed a rise in serum Ca levels (Kumar *et al*., 2021) and noticed a reduction in Ca level on the seventh day after internal fixation of femur fracture in goats, followed by a significant rise in concentration from day 15 to reach the normal level by day 60 of the healing. The increasing trend of serum calcium level initially attributed to increased osteoclastic bone resorption during the initial stages of fracture healing and during the stage of remodeling, this results in calcium mobilization into blood (Venugopalan, 2009).

An increasing trend in the levels of P compared to day 0 was observed within a group. The above results were in agreement with (Daron, 2013) who noticed a significant elevation of P levels after internal fixation of long bone fractures in goats. That is due to the higher levels of P which are attributed to osteoclastic activity leading to the resorption of dead bone thereby increasing the levels of P (Venugopalan, 2009).

The ALP had significantly higher values among the groups (BPBM, BPNS, BWBM, and BWNS) while FHP was observed higher in group BPBM and group BWBM as compared to other groups. The similar patterns were observed for THP. The similar observations were noticed by (Kumar *et al*., 2021) who observed biochemical changes during the healing of long bone fracture in goats along with an increased level of ALP (Singh *et al*., 2008) noticed that the pattern of alteration was the same with fiberglass and plaster of Paris when used for external immobilization of long bone fracture in goats.

With gradual increments, higher levels of serum ALP were observed in groups BPBM and BWBM as compared to groups BPNS and BWBM in a mean time of 45 days. This was significant increase in ALP level. The rise in the ALP level is due to increased osteoblastic activity. Osteoblast releases a large quantity of ALP that participated in the formation of bone matrix and its mineralization and also due to the property of bone marrow aspirate to induce osteoblastic proliferation and rise in the activity of ALP (Zhang *et al*., 2011).

This trend of gradual elevation of THP and FHP up to day 45 was also found. Several studies have also mentioned the increased levels of urinary excretion of FHP and THP following fractures as compared to healthy individuals (Veronesi *et al*., 2013). That is due to the fact that the actual phase of resorption continues for 50 days due to the residual elevation being a result of ongoing collagen synthesis (Vilquin and Rosset, 2006). Hydroxyproline is found mainly in collagen fibers and contains hydroxyproline which is about 13% of the amino acid collagen contents (Çetinkaya *et al*., 2012). As a result of post-translational hydroxylation which occurs within the peptide chain, hydroxyproline is derived from proline. In the consequence of the degradation of collagen, FHP is released which cannot be reutilized for the synthesis of collagen (Purohit *et al*., 1984). Furthermore, about 50% of collagen exists in the bone where its turnover may be faster than the soft tissues which are eliminated in urine therefore; it is considered a bone resorption marker (Brighton and Hunt, 1991). Further studies have also observed increased levels of urinary FHP and THP following fractures as related to healthy animals (Coulibaly *et al*., 2010). Sero-bio-chemical markers for bone healing have been reflected as an assisting diagnostic or prognostic technique for monitoring the process of fracture healing (Al-Sobayil *et al*., 2020). Measurement of serobiochemical markers for bone healing during the process of fracture healing could augment the accuracy of the assessment of the bone healing stage (Coulibaly *et al*., 2010; Cox *et al*., 2010). Our results showed a significant increase in the levels of ALP, FHP, and THP in groups BPBM and BWBM compared to the other groups. This is associated with the stimulation of osteoblasts which plays a pivotal role in the active synthesis and maturation stage of the bone extracellular matrix during the healing process of the fracture (Al-Sobayil *et al*., 2020). In conclusion, BMA has an encouraging osteogenic effect on metacarpal and metatarsal repair of fracture in goats, especially when used in combination with bone plating and bone wiring.

## CONCLUSION

In summary, we observed that the healing process of the metacarpal and metatarsal fractured bones was reduced by bone plating, and bone wiring was ameliorated with the application of multiple BMA. We proposed further studies on larger cohorts. BMA may be used as supportive therapy to enhance the healing process of fractures in goats.

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*IRB approval*

The current research study was approved by Advanced Studies and Research Board of the University of Veterinary and Animal Sciences Lahore, Pakistan in its 50th meeting held on 08-02-2019, and was notified by Directorate of Advanced Studies Vide Letter No. DAS/ 537 dated 05-03-2019.

*Ethical statement*

The present study protocol was approved by the ethical review committee of University of Veterinary and Animal Sciences Lahore, Pakistan Vide Letter No. DR/27 dated 10-01-2019.

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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